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Evolution of acidity of honeys from continental climates: Influence of induced granulation

María M. Cavia [a], Miguel A. Fernández-Muiño [a], Sara R. Alonso-Torre [a], José F. Huidobro [b], María T. Sancho [a,*]

[a] Universidad de Burgos, Área de Nutrición y Bromatología, Departamento de Biotecnología y Ciencia de los Alimentos, Facultad de Ciencias. Plaza Misael Bañuelos García s/n, E-09001 BURGOS (Castilla y León), Spain
[b] Universidad de Santiago de Compostela, Área de Nutrición y Bromatología, Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Farmacia, E-15782 SANTIAGO DE COMPOSTELA (Galicia), Spain

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Abstract

On 35 unheated honeys coming from an area with typical Continental climate, we studied the evolution of pH, free acid, lactones and total acidity throughout 30 months, as well as the influence of induced granulation on acidity evolution. All samples were stored at room temperature and analyzed, in duplicate, each 5 months. We observed that storage had an effect on pH and free acid values. On the contrary, induced granulation did not show any influence on these parameters. In the end of the study, pH average had decreased down to 5%. After 20 months from harvesting, the vast majority of honeys showed a constant increase of free acid, although until 30 months, none sample exceeded the limit of 50 meq/kg. A direct relationship was found between pH and free acid within each time of analysis. Both storage and induced granulation had an effect on honey lactones, parameter that steadily decreased after 20 months, and oscillated less markedly in samples subjected to induced granulation. For honeys from continental climates, taking free acid and lactones into account, 20 months could be proposed as best before period "once opened". Neither storage nor induced granulation had an effect on total acidity, which hardly varied within 30 months storage. We found significant relationships between initial values of free acid and lactones and the values of these parameters at each time of analysis, meaning that aging of these honeys due to acidity could be predicted.

Keywords: Honey; pH; Free acid; Lactones; Total acidity; Best before period once opened

1. Introduction

Honey acidity is mainly due to organic acids whose quantity is lower than 0.5%. Acidity contributes to honey flavour, stability against microorganisms, enhancement of chemical reactions, and antibacterial and antioxidant activities (White, 1975; Faraji-Haremi, 1976; Molan & Russell, 1988; Bogdanov, 1997; Weston, Mitchell, & Allen, 1998; Gheldof, Wang, & Engeseth, 2002). Gluconic acid, resulting from the action of honey's glucose oxidase on glucose, provides the major contribution to acidity and is in equilibrium with gluconolactone (Stinson, Subers, Petty, & White, 1960; White, 1979). Other organic acids together with inorganic anions also contribute to acidity (White, 1975; Cherchi, Spanedda, Tuberoso, & Cabras, 1994; Mato et al., 1997).

Honey pH depends on both the ionised acids of this food and mineral elements and influences microorganisms development, enzymatic activity and texture, among other properties (White, 1962, 1975; Chandler, Fenwick, Orlova, & Reynolds, 1974; Estupiñán, Sanjuán, Millán, & González-Cortés, 1998).

* Corresponding author. Tel.: 34 947 258 813; fax: 34 947 258 831.
E-mail address: mtsancho@ubu.es (M.T. Sancho).

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Free acid is one of the most important parameters for honey quality control that is included within European composition criteria for this foodstuff. According to the European Council Directive, free acid cannot exceed 50 meq/kg for honey in general and cannot exceed 80 meq/kg for baker's honey (OJEC, 2002). Honey's free acid is provided by all free acids as a whole (White, 1978) and can vary widely. Many authors reported that free acid increases with time, as well as during fermentation, because honey sugars and alcohols transform into acids by honey yeasts (Gonnet, 1965; White, 1975; Huidobro & Simal, 1984; Jiménez, Mateo, Huerta, & Mateo, 1994; Bath & Singh, 2000).

Lactones determination is interesting because their hydrolysis increases free acid (White, Petty, & Hager, 1958). Honey lactones vary irregularly (Gonnet, 1965; Krauze & Krauze, 1991; Jiménez et al., 1994). Total acidity is the sum of free acid and lactones.

It is widely known that honey tends to crystallize. Natural crystallisation of honey is usually an unwelcome process. On the one hand, honey texture usually gets worse. On the other hand, an upper liquid phase poor in sugar content can lead to fermentation. In order to avoid these problems, induced granulation appears to be one of the best alternatives. This process consists on seeding a liquid honey with 10% finely crystallized honey at low temperature, so that crystals act as nuclei for growth. The resulting honey is creamy, smooth and very pleasant to taste.

Nowadays, best before periods "once opened" are of particular interest in order to know the period of time in which a given food keeps more or less its original quality, once the jar has been opened. Best before dates are usually established after accelerated assays of aging, and seldom under real conditions of storage, at room temperature. With regard to honey, as far as we know, none paper has been published about any study of best before dates, even though commercially purchased honeys are labelled with best before dates that usually vary from 1 to 3 years. None study has been either developed on honey in order to establish best before periods once opened, that in our opinion, would be more interesting.

The purposes of this study have been first of all, to research, throughout 30 months, the evolution of acidity types in honeys stored at room temperature, coming from a typical continental climate area; secondly, to research the influence of induced granulation on acidity evolution. Finally, to attempt to know the best before period once opened for these honeys regarding acidity, on the basis of both stability data of acidity, and the moment starting on which, free acid begins to increase steadily.

2. Materials and methods

2.1. Samples

This study has been carried out on 35 unheated samples of honey harvested in Burgos, a Spaniard area with typical Continental Climate. Among other parameters, moisture and water activity of all samples were previously analyzed (Cavia, 2002; Cavia, Fernández-Muiño, Huidobro, & Sancho, 2004). Each sample (1 kg) was divided into two aliquots of 500 g and aseptically bottled. One of the aliquots was labelled as "A" and was directly stored. In the second one, labelled as "B", crystallisation was induced by seeding with 10% of finely crystallised honey. In order to achieve the best conditions for the induced granulation, moisture percentage of this finely crystallised honey was lower than 18.5% and glucose/water ratio was higher than 1.80 (Gonnet, 1965). pH and acidity of this finely crystallised honey were analysed, as well. All 70 samples (35 "A" and 35 "B") were kept in darkness and stored at room temperature (between 15 °C and 25 °C).

The analyses have been carried out over 3 years, each 5 months, at 5, 10, 15, 20, 25 and 30 months. The first 5 months after harvesting were necessary to collect and select the samples for induced crystallization.

2.2. Methods

pH, free acid and lactones, were determined according to the procedure proposed by White (White et al., 1958; White, 1962), official "Final Action" (method 962.19) in the AOAC (2002), by using a potentiometer titrator (751 GPD Titrinio METROHM) provided with a specific electrode to measure honey acidity (Metrohm No. 6.0222.100) because other similar electrode (Methrom No. 6.0228.000), previously assayed by us, gave imprecise and irreproducible results. All samples were analysed in duplicate. Determination of acidity was repeated for every sample until the difference between the results of duplicate analysis of each sample (obtained by the same procedure under the same conditions in rapid succession) did not exceed the maximum value given in the precision tables of Harmonised Methods of the European Honey Commission (Bogdanov, Martin, & Lüllmann, 1997).

Statistical analysis of the data was carried out with Statgraphics plus software 4.0 (1997). Multifactor analysis of variance (ANOVA) was performed to examine the effects of storage and induced granulation on pH and acidity and to estimate the statistically significant relationship between different parameters.

3. Results and discussion

Fig. 1 shows the average pH of the samples and its evolution over 30 months. pH average of samples "A" kept constant during the first 15 months. Then, it dropped down to 5%. In "B" samples pH kept constant 20 months, and then, it also dropped down to 5%. Jiménez et al. (1994) showed that pH was very stable over two years of storage. Bath and Singh (2000) observed that pH decreased with storage, in honeys that had been heated before storage.

Until 30 months from harvesting, none sample showed a free acid value higher than the limit of 50 meq/kg (OJEC,

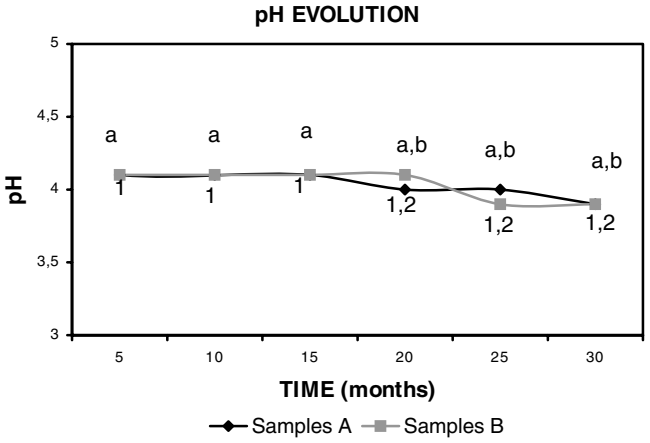

Fig. 1. Evolution of pH of samples A and B.

2002). At 30 months 2 "A" samples and 1 "B" sample showed values higher than 50 meq/kg. Fig. 2 shows the evolution of free acid average of samples "A" and "B" and the significant differences that were found between values at different months. We observed that free acid remained almost constant from the beginning to 15 months, with a slight tendency to increase. Between 15 and 20 months, free acid of all samples decreased, from an average of 27.9 ± 8.6 meq/kg to an average of 24.1 ± 9.7 meq/kg in "A" samples, and from an average of 28.0 ± 7.3 meq/kg to an average of 24.2 ± 8.3 meq/kg in "B" samples. After 20 months, all "A" honeys and 94% "B" honeys showed a constant increase of free acid. Jiménez et al. (1994) observed a slight increase of free acid in honeys stored 2 years. Bath and Singh (2000) also observed an increase of free acid throughout 12 months, in honeys that had been heated before storage.

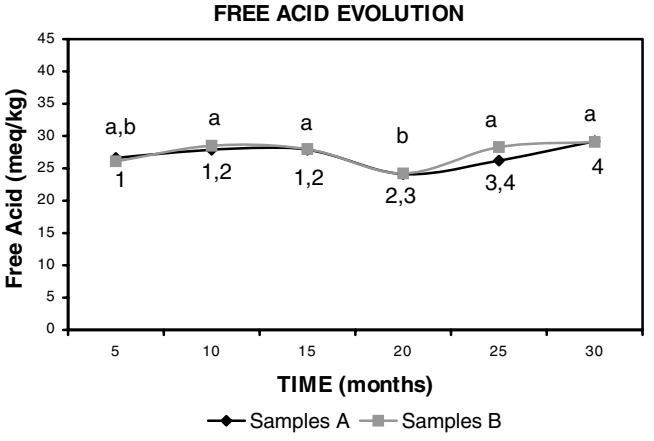

Fig. 2. Evolution of free acid of samples A and B.

Table 1
Analysis of variance of data of pH, free acid, lactones and total acidity in samples A and B

Source	DF	Induced granulation 1	Time storage 5	Interaction 5
pH	SS	0.0800952	2.00305	0.0679048
	F	1.02	5.08*	0.17
Free acidity	SS	14.8972	1068.42	69.923
	F	0.19	2.67*	0.17
Lactone acidity	SS	134.753	1416.12	180.138
	F	11.32*	23.80*	3.03*
Total acidity	SS	78.6934	1010.55	95.578
	F	0.67	1.71	0.16

DF = Degrees of Freedom.
SS = Sum of Squares.
F = *F*-ratio of ANOVA.
* Significant differences at *P*-value < 0.05.

Free acid changes are likely to occur because such enzymes as glucose oxidase, that are active after honey harvesting, modify honey composition. Montes (1996) suggested that both levulinic and formic acids could come from hydroxymethylfurfural, thereby increasing free acid.

Statistical analysis made it clear that storage had an effect on pH and free acid values. On the contrary, induced granulation did not show any influence on these parameters (Table 1).

White (1975) did not find a direct relationship between pH and free acid of honey. Nevertheless, in this study we have found a significant statistic relationship (Table 2)

Table 2
Relationships between pH values and free acid values

	Sample A		Sample B	
	Model[a]	Correlation coefficient (*r*)	Model[a]	Correlation coefficient (*r*)
5 months	FA = −17.5618 + 10.7462 × pH	0.3902*	FA = −18.3744 + 10.8434 × pH	0.3941*
10 months	FA = −30.9971 + 14.2991 × pH	0.4455**	FA = −40.9541 + 17.0339 × pH	0.5436**
15 months	FA = −30.2217 + 14.1362 × pH	0.4674**	FA = −26.2442 + 13.2737 × pH	0.5076**
20 months	FA = −33.4716 + 14.2208 × pH	0.4199*	FA = −42.1909 + 16.3446 × pH	0.5143**
25 months	FA = −43.4002 + 17.3759 × pH	0.4433**	FA = −34.8813 + 16.0201 × pH	0.4961**
30 months	FA = −57.8692 + 22.1319 × pH	0.5441**	FA = −39.767 + 17.6684 × pH	0.5055**

[a] FA is free acid.
* Statistically significant relationship at *P*-value < 0.05.
** Statistically significant relationship at *P*-value < 0.01.

between both parameters in both "A" and "B" samples within each time of analysis.

Fig. 3 shows the evolution of average lactones of samples "A" and "B" and the significant differences that were found between values at different months. From 5 to 10 months, lactone acidity of "A" and "B" samples kept constant, although it tended to decrease slightly. From 10 to 15 months lactones of both "A" and "B" samples increased, more markedly in "B" samples. Then, lactones decreased until an average value similar to the initial mean value. Even though trends of evolution for "A" and "B" samples were similar, lactones of "A" samples showed more oscillations than lactones of "B" samples. Gonnet (1965) observed that free acid increased with storage; on the contrary, lactones did not change following the same pattern. Krauze and Krauze (1991) obtained a more marked rise in lactone than in free acid with honey storage. Jiménez et al. (1994) observed a slight increase of honey lactones throughout two years. All these studies seem to make it clear that lactones can change depending on the chemical balance between them and their respective acids.

Statistical analysis made it clear that both storage and induced granulation had an effect on honey lactones (Table 1).

Fig. 4 shows the evolution of total acidity of samples "A" and "B" and the differences that were found between values at different months. In both "A" and "B" samples total acidity hardly varied within 30 months storage. Trends of total acidity evolution for both "A" and "B" samples were similar.

Statistical analysis made it clear that neither storage nor induced granulation had an effect on total acidity (Table 1).

Seeing that free acid and lactones were modified by storage, we calculated the variation rate of these parameters (Table 3). Induced granulation proved to have an effect on the evolution rate of both free acid and lactones.

The increase of free acid variation rate was different between "A" and "B" samples at 10 months, being higher

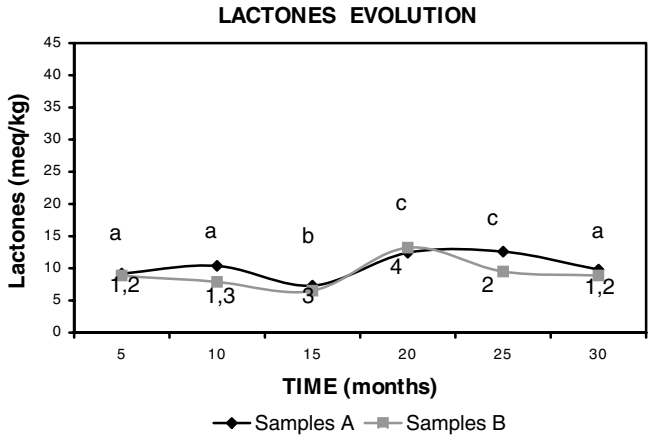

Fig. 3. Evolution of lactones of samples A and B.

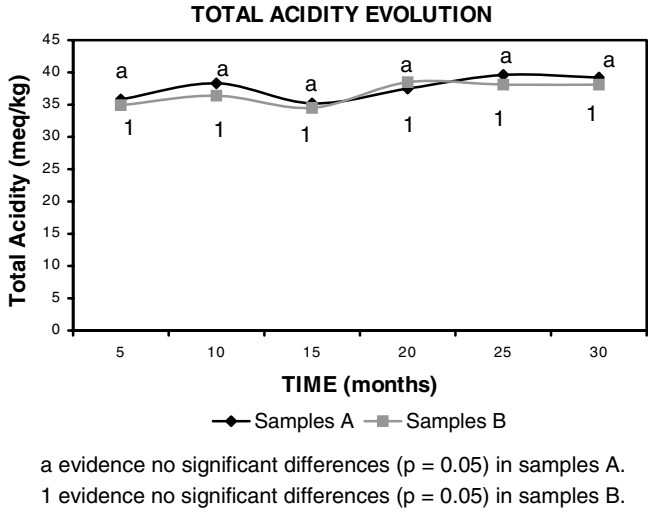

Fig. 4. Evolution of total acidity of samples A and B.

in "B" samples. At 25 months, we observed a slight decrease of free acid variation rate in "A" samples, whereas there was an increase of free acid variation rate in "B" samples, so that regarding free acid, induced granulation did not seem to delay honey aging, even though it did not accelerate it either, because in the end of the study (at 30 months) values of free acid for both "A" and "B" samples were statistically similar.

Lactones variation rate was also different in "A" and "B" samples. At 10 months, lactones of "A" samples increased, whereas lactones of "B" samples slightly decreased. At 25 months, lactones increased in both "A" and "B" samples but more markedly in "A" samples. With regard to lactones, induced granulation seemed to delay

Table 3
Variation rate of free acid and lactones in samples A and B*

	Free acid	Lactones
10 months		
A	5.4 ± 0.79^{a}	28.6 ± 9.3^{a}
B	10.7 ± 1.25^{b}	-1.1 ± 6.8^{b}
15 months		
A	6.2 ± 1.2^{a}	-9.1 ± 9.0^{a}
B	9.5 ± 1.56^{a}	-23.4 ± 6.2^{a}
20 months		
A	-10.7 ± 1.26^{a}	59.0 ± 13.3^{a}
B	-7.4 ± 1.29^{a}	68.2 ± 9.92^{a}
25 months		
A	-3.0 ± 1.6^{a}	67.5 ± 16.4^{a}
B	9.2 ± 1.55^{b}	22.7 ± 8.9^{b}
30 months		
A	9.7 ± 1.45^{a}	22.7 ± 9.5^{a}
B	12.6 ± 1.34^{a}	14.3 ± 8.2^{a}

* Mean values of variation rate of total samples. Data expressed as means ± SEM (Standard error of mean). Values in the same quadrate that are followed by a different roman letter ([a] and [b]) are significantly different (P-value < 0.05) using ANOVA.

Table 4
Relationships between initial value of free acid and lactones and showed values at different analysis

Sample A	Free acid[a]	Lactones[b]
10 months	FA2 = 1.6311 + 0.9863 × FA1 $r = 0{,}9886^{*}$	LA2 = 4.4066 + 0.6549 × LA1 $r = 0{,}8201^{*}$
15 months	FA3 = 3.6694 + 0.9096 × FA1 $r = 0{,}9777^{*}$	LA3= 1.4654 + 0.6342 × LA1 $r = 0{,}6827^{*}$
20 months	FA4 = −3.0355 + 1.0197 × FA1 $r = 0{,}9770^{*}$	LA4= 6.584 + 0.6320 × LA1 $r = 0{,}7330^{*}$
25 months	FA5 = -2.9810 + 1.0972 × FA1 $r = 0{,}9684^{*}$	LA5 = 8.8069 + 0.4116 × LA1 $r = 0{,}5403^{*}$
30 months	FA6 = 0.7190 + 1.0693 × FA1 $r = 0{,}9639^{*}$	LA6 = 4.2176 + 0.6037 LA1 $r = 0{,}6976^{*}$

[a] FA is free acid. Number mark analysis carried out (i.e., two mark analysis at 10 months, three analysis at 15 months).
[b] LA is lactones.
[*] Statistically significant relationship at P-value < 0.01.

Table 5
Relationships between initial value of free acid and lactones and showed values at different analysis

Sample B	Free acid[a]	Lactones[b]
10 months	FA2 = 4.2311 + 0.9309 × FA1 $r = 0{,}9664^{*}$	LA2 = 3.3968 + 0.5052 × LA1 $r = 0{,}7017^{*}$
15 months	FA3 = 6.1381 + 0.8380 × FA1 $r = 0{,}9584^{*}$	LA3 = 0.1461 + 0.7194 × LA1 $r = 0{,}6513^{*}$
20 months	FA4 = 7.619 + 0.8767 × FA1 $r = 0{,}9563^{*}$	LA4 = 8.0843 + 0.5795 × LA1 $r = 0{,}7327^{*}$
25 months	FA5 = 10.6335 + 0.90132 × FA1 $r = 0{,}9538^{*}$	LA5 = 5.1432 + 0.4976 × LA1 $r = 0{,}6515^{*}$
30 months	FA6 = 10.7154 + 0.9292 × FA1 $r = 0{,}9635^{*}$	LA6 = 5.1381 + 0.4204 × LA1 $r = 0{,}6397^{*}$

[a] FA is free acid. Number mark analysis carried out (i.e., two mark analysis at 10 months, three analysis at 15 months).
[b] LA is lactones.
[*] Statistically significant relationship at P-value < 0.01.

aging because during storage, lactones of "A" samples were modified more markedly than lactones of "B" samples. However, we checked that this delay of aging was not significant because in the end of the study, lactone acidity values of "A" and "B" samples were similar.

Statistical analysis made it clear that neither moisture nor water activity had an effect on both pH and acidity evolution.

Without being a purpose of our work, we found significant relationships between initial values of free acid and lactones and the values of these parameters at each time of analysis (Tables 4 and 5). These relationships were significant for both "A" and "B" samples. This means that it would be possible to know, at a given time, the value of free acid or lactones on the basis of the initial value of these parameters. Krauze and Krauze (1991) found similar correlations for free acid but not for lactones. These authors claimed that lactones of fresh honeys were not related with lactones of old honeys. Our opinion is that those possible relationships are very interesting fields to research. If same relations are found in different honeys from different botanical sources, geographical origins, and harvesting years, aging of honey due to acidity could be predictable.

According to our results, it seems that after 20 months free acid increases continuously, whereas lactone acidity decreases, so that taking acidity into account, 20 months could be proposed, for honeys coming from Continental climates, as best before period once opened. Induced granulation does not appear to significantly influence aging due to acidity.

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